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KAR3 encodes a kinesin-like motor protein that has a wide variety of roles in the budding yeast *Saccharomyces cerevisiae*. Genetic bypass studies and a mutant hunt are being done to discern the nature of the *kar3* meiotic arrest and to find genes involved in the checkpoint function that mediates the arrest. Checkpoints are important regulatory mechanisms used by cells to prevent aberrant cell cycle divisions such as those observed in cancerous cancerous cells. In addition Kar3p functions are thought to be mediated by interaction with a number of kinesin associated proteins (KAPs) including Cik1p and Vik1p. We have explored the roles of these KAPs in meiosis. Cik1p is essential for meiosis, while Vik1p has a very minor role. These data are consistent with Cik1p and Kar3p acting together to perform the essential role of Kar3p in meiosis. We have also localized Kar3p, Cik1p,and Vik1p during meiosis.

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INTRODUCTION:

We use the budding yeast *Saccharomyces cerevisiae* as a model organism to study the meiotic cell cycle. Yeast cells deleted for a gene encoding a motor protein called Kar3 arrest during early meiosis (Bascom-Slack and Dawson 1997) in what we believe to be a checkpoint mediated fashion. The purpose of this research is to identify checkpoint genes involved in the initiation and maintenance of the *kar3* meiotic arrest. We have searched for mutations in genes that would allow *kar3* mutants to proceed through meiosis. In addition we have explored the meiotic roles of genes known to mediate the function of Kar3p during other cellular processes.

BODY:

Training: Since the inception of the grant I have completed all of my required classes and have passed my qualifying exam. I have taught small groups (14-16 students) in Molecular Biology Classes for first year medical students (as 'lead T.A.') for three years and I have been a teaching assistant at the Cold Spring Harbor Yeast Genetics Course (1999). I have also taught (three 8-week sessions)"wet-labs" for medical and dental students where I help students master basic microbiological techniques. I have presented this research at yearly departmental research reports, and weekly lab meetings. In addition I regularly attend Boston Area Yeast Meetings and Departmental Seminars (thrice weekly). I attended the Gordon Research Conference on Meiosis twice at which I presented posters.

Research: 1a. Identification of genes involved in the meiotic arrest of *kar3* mutants: Mutant Hunt. A mutant hunt described in the proposal was carried out. The Snyder Library (Burns *et al.* 1994) was used to generate 35,130 mutants (over five times the number of genes in the yeast genome) in a *kar3* mutant strain. After generating diploids homozygous for both the new mutation and *kar3*, these diploids were replica plated onto sporulation medium containing X-gal in order to identify in frame insertions in genes that are expressed during sporulation (since only 1/6 of insertions will be in the correct orientation and frame the number of genomes screened is reduced to 0.9). These mutants were screened with fluorescent microscopy to identify mutations that allow nuclear division of *kar3* cells during meiosis. Ten candidates were reproducibly allowed meiotic progression beyond the point of the *kar3* meiotic. Five mutant candidates have been identified using the protocol specified by the Yale Genome Analysis Center (instructions are available on line at http://ygac.med.yale.edu/). These include two unknown open reading frames (YJL017W, YGR093W) and three known genes:

1: *KAP95*- encodes for an essential nuclear import factor (Iovine and Wente 1997; Seedorf and Silver 1997). The insertion site (in frame at codon 390) must leave a partially functional protein product. This

gene has been shown by two genomic studies (available on the web) to be expressed during meiosis. It is not obvious why *kar3* mutants are suppressed by a second mutation in *kap95*, but one could hypothesize that Kap95p is necessary to import nuclear factors (checkpoint proteins?) involved in the regulatory arrest of *kar3* mutants.

2. *CHS1*- encodes a sporulation gene involved in spore coat formation (Shaw *et al.* 1991). It is not obvious why the *kar3* arrest is suppressed by this mutation. We are in the process of generating double mutants to recapitulate the bypass phenotype.
3. *NEW1*- encodes a little-characterized protein that is nuclear and contains an ATP-binding cassette (Derkatch *et al.* 2001). Meiotic expression of this gene has been shown by a genomic system. We have generated deletions of *new1*, and found that they sporulate at wild type levels with no statistical reduction in viability. We are in the process of generating double mutants to recapitulate the bypass phenotype.

Mutant hunt conclusions: We have found five individual genes that bypass the *kar3* meiotic arrest in initial and secondary screens. We are now generating deletion mutations of these genes in fresh strains and trying to recapitulate the bypass phenotype. None of the genes we found were obvious candidates, so we have spent more time analyzing the *kar3* arrest with the mix and match strategy, see Body section 1b.

1b. Mix and Match. We introduced mutations into genes in a *kar3* mutant strain to determine if these genes are important for the meiotic arrest. Genes chosen were known checkpoint genes or other genes involved in microtubule or meiotic regulation. We hypothesize that if the gene ("gene X") is involved in the establishment of maintenance of the *kar3* arrest than a double mutant of *kar3* *geneX* will be able to proceed further into meiosis than *kar3* mutants. Double mutants and control strains are generated than induced to undergo meiosis. At late time points when wild type controls cells have finished meiosis. We

cytologically (DAPI, anti-tubulin staining) assay double mutants and control strains for meiotic progression.

Completed Mix and Match experiments and their result:

-*spo11* (a gene that encodes for the endonuclease that makes double stranded breads and effects meiotic timing and checkpoint activation (Keeney *et al.* 1997)) - bypass

-*spo13* (a meiotic regulatory gene of undefined function, but known to suppress the meiotic arrest of some mutations (Ogawa *et al.* 1995)) – no bypass

-*rad17* (a recombination checkpoint (Lydall *et al.* 1996)) – no bypass

-*mek1* (a synaptonemal complex component that is involved in meiotic regulation (Bailis and Roeder 2000))- no bypass

-*red1* (a synaptonemal complex component that is involved in meiotic regulation (Bailis and Roeder 2000)) – no bypass

-*pch2* (a meiotic "pachytene-checkpoint" gene (San-Segundo and Roeder 1999)) – no bypass

Future Mix and Match experiments: Spindle checkpoint genes. *kar3* has been shown to be synthetically lethal with mutations in the spindle checkpoint genes (Roof *et al.* 1991). This implies that a microtubule or chromosome segregation defect in *kar3* mutants is monitored by the spindle checkpoint, and that the function of this checkpoint in *kar3* mutants is essential. The spindle checkpoint genes (*MAD1-3* in *S. cerevisiae* (Shonn *et al.* 2000) and *BUB1* in *S. pombe* (Schwab *et al.* 2001)) have recently been implicated as having a role in meiotic chromosome segregation. We think that there is a strong possibility that *kar3* mutants arrest because of the spindle checkpoint. To circumvent the mitotic synthetic lethal phenotype we have built put *MAD2* under the control of a tetracycline-inducible promoter (Belli *et al.* 1998). We will generate *kar3 mad2* double mutants in the presence of tetracycline in a strain bearing the inducible *MAD2* construct. This strain will be grown in the presence of tetracycline (*MAD2*- expression) and then washed and placed in sporulation medium in the absence of tetracycline; a control strain will be sporulated in the presence of tetracycline. We will then cytologically assay the meiotic expression of these strains. We have at this point generated *mad2* mutants and shown that their meiotic defect (reduced spore viability) is rescued by the presence of the

inducible *MAD2* construct + tetracycline. We have also shown that the inducible *MAD2* construct rescues certain mitotic defects of *mad2* mutants (sensitivity to microtubule destabilizing chemicals). Therefore, we believe that our inducible *MAD2* construct produces functional Mad2p, and will be suitable for our mix and match experiment. This result would be novel because the spindle checkpoint has been shown to act at the metaphase to anaphase transition in mitotic cells, and in meiosis the checkpoint may act in prophase. A positive result (*mad2 kar3* double mutant proceeding past prophase I) would give us insight into the differences between meiosis and mitosis and into the way that checkpoints work.

Mix and match conclusions: From these data we conclude that *kar3* mutants are capable of meiotic division (*kar3 spo11* mutants form binucleate cells with normal looking spindles), and are likely to arrest because of a checkpoint rather than a physical inability to make a meiotic spindle. We do not know why *spo11* allows suppression of *kar3* mutants because mutations in *spo11* are known to have pleiotropic effects on meiosis. Also, since the *rad17 kar3* cells still arrest we are conclude that the lowered amounts of mature recombination products are not solely responsible for the *kar3* meiotic arrest.

2. Characterization of meiotic phenotypes of *cik1* and *vik1* mutants, and the localization of tagged versions of Cik1p, Vik1p and Kar3p. *CIK1* and *VIK1* encode similar proteins that mediate the functions of Kar3p during various cellular processes (Manning *et al.* 1999). It has been shown that Cik1p directly interacts with Kar3p is essential for the function of Kar3p during karyogamy (nuclear fusion in mating cells), and that *cik1* mutants share many phenotypes with *kar3* mutants in mitotic cells (Page *et al.* 1994; Page and Snyder 1992). Vik1p has been shown to interact with Kar3p during mitosis, but not during karyogamy. The roles of Cik1p and Vik1p during meiosis have not been thoroughly characterized, although both of these genes are expressed in meiosis (the Transcriptional Program of Sporulation, (Chu *et al.* 1998)). We have assayed meiotic phenotypes of *cik1* and *vik1* mutants in meiosis. We are assaying meiotic phenotypes commonly used to characterize meiotic defects.

2a. Cik1p studies: We have generated deletions mutations of *cik1* and characterized the meiotic phenotypes conferred by the absence of this gene. In most cases *cik1* mutants behave like *kar3* mutants in meiosis. I have briefly listed some of our findings below, *cik1* mutant meiotic phenotypes are similar to published data for *kar3* mutants (Bascom-Slack and Dawson 1997), except where noted. We also cloned *CIK1* (using the protocol of (Page and Snyder 1992)) and made 13xMyc-tagged versions of Cik1p (Longtine *et al.* 1998) to further analyze the role of Cik1p in meiosis.

-*cik1* mutants do not form spores.

-*cik1* mutants arrest in meiosis with monopolar tubulin arrays.

-*cik1* mutant prophase microtubule arrays are slightly longer than those of wild type prophase cells, and significantly shorter than those of *kar3* mutant.

-*cik1* mutants are severely deficient in meiotic recombination.

-*cik1* mutants show incomplete and abnormal synaptonemal complex formation.

-*cik1 rad17* mutants remain arrested in meiosis I.

-*cik1 pch2* mutants remain arrest in meiosis I.

-*cik1 spo13* mutants form binucleates at levels equal to isogenic *spo13* mutants. This is distinctly different than the *kar3 spo13* double mutants which remain arrested in meiosis I. This suggests Kar3p has Cik1p-independent functions during meiosis.

-(mitotic phenotype) *cik1* is synthetically lethal with checkpoint genes mad1 and mad3.

-Functional Myc-tagged fusions of Cik1p were made and the localization of Cik1p was assayed throughout meiosis. We found that Cik1p showed three patterns of localization:

a. Spindle Pole Body (SPB, yeast's microtubule organizing center –like organelle) localization, confirmed by colocalization experiments with Tub4-GFP (yeast's gamma tubulin homolog, a known SPB component (Marschall *et al.* 1996))
b. general nuclear localization that persisted throughout meiosis I and meiosis II

c. spindle midzone-like localization. Cik1p was seen between the nuclei of late anaphase I cells, a pattern shown often described for proteins that associate with kinetochores (Arnaud *et al.* 1998; Chan *et al.* 1998; Zeng *et al.* 1999). This midzone pattern of localization was interestingly not observed by Kar3p. This strongly suggests that at least some Cik1p-Kar3p complex disassociates at anaphase I.

Cik1 conclusions: These data are consistent with the model that Cik1p interacts with Kar3p to mediate its essential role in meiosis I; however, Kar3p clearly has Cik1p independent functions.

2b. Vik1p studies: We have generated deletions mutations of *vik1* and characterized the meiotic phenotypes conferred by the absence of this gene. *vik1* mutants have week meiotic phenotypes (Manning *et al.* 1999). We also cloned *VIK1* and made 13xMyc-tagged versions of Vik1p to further analyze the role of Vik1p in meiosis.

-*vik1* mutants sporulate at wild type levels.

-*vik1* mutants have spore viability that is lower than isogenic wild type strains.

-*vik1* mutants have prophase nuclear microtubule lengths indistinguishable from wild type prophase nuclear microtubules.

-*vik1* mutants do not generate spore products with elevated frequencies of aneuploidy compared with isogenic wild type strains. *vik1* mutants, therefore, do not have elevated levels of meiosis I nondisjunction. The lowered viability of *vik1* spores is not due to elevated levels of MI nondisjunction.

-*vik1* mutants were assayed cytologically for elevated levels of meiosis II nondisjunction. We found that *vik1* mutants do not have elevated levels of MII nondisjunction.

-*vik1* mutants have elevated levels of vegetative chromosome loss. This elevated chromosome loss frequency contributes to the lowered viability of *vik1* meiotic products.

-Overexpression of *VIK1* does not suppress the meiotic defect of *cik1* mutants consistent with the hypothesis that the Vik1p-Kar3p complex does not share redundant roles with the Cik1p-Kar3p complex.

-*vik1 cik1 spo13* mutants form viable dyads (similar to the *cik1 spo13* phenotype mentioned above). This shows that in a *spo13* mutant background Vik1p is not substituting for Cik1p.

-We constructed functional Myc-tagged fusions of Vik1p and showed the localization of Vik1p throughout meiosis. We found that Vik1p was found during both Meiosis I and Meiosis II and that it colocalizes with spindle pole bodies.

Vik1 conclusions: These data support that model that Vik1p plays a minor or redundant role in meiosis.

2c. Kar3p studies: Further experiments were done to characterize the kar3 meiotic arrest and 13xMyc tagged versions of Kar3p were constructed to aid in localization experiments.

-*kar3* mutants have greatly (approximately 2-fold) elongated prophase nuclear microtubule arrays.

-*kar3* mix and match experiments (see section 1B above).

-Functional Myc-tagged versions of Kar3p were found to have 3 localization patterns:

a. Kar3p was found to colocalize with SPBs (as seen in vegetative cells), and to colocalize with known SPB proteins.
b. Kar3p was shown to have a general nuclear localization throughout meiosis.
c. Kar3p was found to localize faintly along the meiotic spindle. Kar3p was seen at all stages of meiosis.

Kar3p conclusions: These data are consistent with Kar3p functioning throughout meiosis in association with microtubule components. The general nuclear localization of Kar3p is consistent with a DNA interaction. Kar3p has been implicated to interact with centromeres of mitotic chromosomes (Middleton and

Carbon 1994). We have unsuccessfully tried to colocalize Kar3p with a known kinetochore component protein (Ndc10-GFP) (Doheny *et al.* 1993; Goh and Kilmartin 1993; Jiang *et al.* 1993). From our negative data, we cannot conclude whether or not Kar3p interacts with DNA in meiosis.

Kar3p Model: We hypothesize that Kar3p and Cik1p act together in meiosis I for an essential meiotic function. The phenotypes displayed by *kar3* (and *cik1*) mutants are consistent with the absence of recombination in meiosis. Yet why would a motor protein be involved in recombination? Our model is that in the Kar3p-Cik1p complex indirectly facilitates recombination by bringing homologous chromosomes together. In prophase of meiosis I chromosomes have been shown to cluster in two ways (centromere clustering and telomere clustering (Trelles-Sticken *et al.* 1999)), both of these ways appear to focus on the unseparated spindle pole bodies of the prophase I microtubule array. We hypothesize that the Kar3p-Cik1p complex associates with DNA (telomeres or centromeres) and marches them in a negative direction toward the SPBs (Kar3p's motor polarity is negative) the result of which is a clustering of chromosomes. This clustering reduces the three dimensional space homologous chromosomes must search in order to find one another. We are now attempting to visualize these chromosome associations in prophase cells and determining if they are dependent upon Kar3p and Cik1p.

Key Research Accomplishments:

- Identification of *kar3* meiotic suppressors.
- The *kar3* meiotic arrest is suppressed by mutations in *spo11*
- The *kar3* meiotic arrest is not suppressed by mutations in *rad17*, *pch2*, *spo13*, *mek1*, or *red1*.
- Cik1p, a Kar3p-binding protein, is essential for meiosis.
- *cik1* mutants do not form spores.
- *cik1* mutants arrest in prophase of meiosis I, with elongated nuclear monopolar tubulin arrays.
- *cik1* mutants have severe recombination defects.
- *cik1* mutants show abnormal synaptonemal complex formation.
- The *cik1* meiotic arrest is not suppressed by mutations in *rad17*, nor *pch2*.
- The *cik1* meiotic arrest is suppressed by *spo13* mutations.
- Cik1p is essential for meiosis I, but not a meiosis II-like division.
- *cik1* mutations are synthetically lethal with *mad1* and *mad3* mutations.
- vik1 mutants are not essential for meiosis.
- Diploid *vik1* mutants experience elevated levels of vegetative chromosome loss.
- *vik1* mutant do not experience elevated levels of meiosis I, nor meiosis II nondisjunction.
- *vik1* prophase nuclear microtubule arrays are indistinguishable from wild type.
- *kar3* prophase nuclear microtubule arrays are highly elongated.
- Vik1p does not have redundant function with Cik1p in meiosis.
- Kar3p meiotic localization.
- Cik1p meiotic localization.
- Vik1p meiotic localization.
- Kar3p, Cik1p, and Vik1p are present in cells in meiosis I and meiosis II.

Reportable Outcomes:

All of the data found on the meiotic roles of Kar3p-interacting proteins Cik1p and Vik1p are found in the following in press article.

Shanks, R.M., Kamieniecki, R.J., and D. S. Dawson The Kar3-interacting protein, Cik1p Plays a Critical Role in Passage through Meiosis I in Saccharomyces cerevisiae. in press - Genetics

The results of "mix and match" experiments on checkpoint regulation in *kar3* and *cik1* mutants will be the topic of an additional paper.

Conclusions:

We have found suppressors of the *kar3* meiotic arrest. This is interesting because some of the suppressors map to uncharacterized genes that could encode potential novel checkpoint genes. We have not fully characterized these genes, and plan to do so in the immediate future. This also shows that *kar3* mutants are able to physically undergo meiosis I, suggesting that *kar3* mutants arrest because of a regulatory (checkpoint) mechanism rather than a physical inability to undergo meiosis. We are in the process of determining whether the spindle checkpoint is important for initiating or maintaining the kar3 meiotic arrest.

We have explored the meiotic phenotypes of known Kar3p-binding proteins (Cik1p and Vik1p). We found that Cik1p is essential for meiosis (a new role for Cik1p) and that *cik1* mutants have meiotic phenotypes that are similar to those of *kar3* mutants. Vik1p has a minor meiotic role and diploid *vik1* mutants have an elevated level of chromosome loss in mitosis.

We have localized Kar3p, Cik1p, and Vik1p. They are nuclear and have microtubule associated localization patterns. Vik1p and Cik1p have distinct localization patterns suggesting that the Vik1p-Kar3p and Cik1p-Kar3p complexes function in different locations consistent with their differing mutant phenotypes. Kar3p and Cik1p both show general nuclear staining as will as association with microtubule organizing centers. This staining pattern is consistent with the DNA associations that are central to our model. Cik1p shows a spindle midzone staining after anaphase I, similar to many kinetochore binding proteins (a phenotype that Kar3p does not share).

We hypothesize that Cik1p and Kar3p act together in prophase I for the essential function of Kar3p. Our model is that the Cik1p-Kar3p complex binds to DNA (centromeres or telomeres) and moves it toward the unseparated microtubule organizing centers of to reduce the area in which homologous chromosomes have to search for one another in the prophase meiotic nucleus. In the absence of this activity homologous chromosomes are unable to find one another and are there for unable to undergo recombination (a phenotype seen in *cik1* and *kar3* mutants). We are in the process of using cytological assays to determine if chromosome pairing is inhibited in *cik1* and *kar3* mutants.

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Appendix 1

Abstract for the 1999 Gordon Research Conference on Meiosis

The Kar3-interacting protein, Cik1p Plays a Critical Role in Passage through Meiosis I

Robert Shanks and Dean Dawson Tufts University Department of Molecular Microbiology, Boston MA.

KAR3 encodes a kinesin-like motor protein that has a wide variety of roles in the budding yeast *Saccharomyces cerevisiae*. Kar3p functions are thought to be determined by interaction with a number of kinesin associated proteins (KAPs) including Cik1p and Vik1p. We are exploring the roles of these KAPs in meiosis. *cik1Δ* mutants display a meiosis I arrest with characteristics similar to those exhibited by *kar3Δ* mutants, including greatly reduced levels of heteroallelic recombination at the *ARG4* locus, and defects in homolog synapsis. In contrast *vik1Δ* mutants form spores at almost wild-type levels. Bypass studies have are being done to discern the nature of the *kar3Δ* and *cik1Δ* meiotic arrests. Mutations in the recombination checkpoint gene *RAD17* have no effect upon the meiotic arrest conferred by *cik1Δ* or *kar3Δ* mutations, suggesting the arrest is not solely mediated by this checkpoint. However, we have found that *cik1Δ pch2Δ* double mutants are able to proceed through the first meiotic division at elevated levels compared to *cik1Δ* mutants, suggesting the arrest is checkpoint mediated. Finally have found that Kar3p may have meiotic roles that do not require Cik1p as *kar3Δ spo13Δ* double mutants fail to progress through meiosis, while *cik1Δ spo13Δ* mutants form spores at levels equivalent to *spo13Δ* mutants alone. Further studies are being done to explore the roles of Kar3p, Cik1p and Vik1p in meiosis.